# Class I PI 3-kinases

# Class I PI 3-Kinases: function and evolution

Nisha Kriplani, Miguel A. Hermida, Euan R. Brown and Nicholas R. Leslie*

*Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot Watt University, Edinburgh, EH14 4AS, UK*

*For correspondence Email: n.r.leslie@hw.ac.uk Tel: 44 131 451 8157

**Abstract**: In many human cell types, the class I phosphoinositide 3-kinases play key roles in the control of diverse cellular processes including growth, proliferation, survival and polarity. This is achieved through their activation by many cell surface receptors, leading to the synthesis of the phosphoinositide lipid signal, $PIP_3$, which in turn influences the function of numerous direct $PIP_3$-binding proteins. Here we review PI3K pathway biology and analyze the evolutionary distribution of its components and their functions. The broad phylogenetic distribution of class I PI3Ks in metazoa, amoebozoa and choanoflagellates, implies that these enzymes evolved in single celled organisms and were later co-opted into metazoan intercellular communication. A similar distribution is evident for the AKT and Cytohesin groups of downstream $PIP_3$-binding proteins, with other effectors and pathway components appearing to evolve later. The genomic and functional phylogeny of regulatory systems such as the PI3K pathway provides a framework to improve our understanding of the mechanisms by which key cellular processes are controlled in humans.

**Keywords**: Phosphoinositide, PI3K, AKT, PTEN, Signal Transduction, Cancer, Kinase, Phosphatase, Evolution, Phylogeny

## Introduction:

All cells use a variety of biochemical mechanisms to control their behaviour in response to changes in their environment, whether they are single cells or those making up a large complex organism. The PI 3-kinase/PTEN signalling network is an important biochemical component in this signal transduction process, and which is best recognized as a regulator of cell growth, proliferation and survival in many cell types. A consequence of these functions is that loss of control of PI3K signalling is a frequent characteristic of human disease, particularly many cancers, in which aberrant activation of PI3K itself or loss of the opposing phosphatase, PTEN, are frequently observed events. In this review, we summarize our current understanding of the pathway, analyze the evolutionary distribution of pathway components and relate this knowledge with its functions in human cells.

## The Phosphoinositide 3-Kinases (PI3Ks)

Phosphoinositide 3-kinases (PI3Ks) are enzymes which phosphorylate membrane phosphoinositide lipids at the 3-hydroxyl group of the inositol ring (Engelman et al., 2006, Vanhaesebroeck et al., 1997a, Whitman et al., 1988). Their lipid products act as cellular signals, directly regulating diverse lipid-binding effector molecules and thus cellular processes including membrane trafficking, cell growth, proliferation, metabolism, migration and polarity (Katso et al., 2001, Martin-Belmonte et al., 2008, Vivanco and Sawyers, 2002). Based on substrate preference and sequence similarity of the catalytic subunit, PI3Ks are classified in three groups, classes I, II and III (Engelman, 2006, Vanhaesebroeck et al., 2001, Vanhaesebroeck, 1997a, Wymann and Pirola, 1998). Class II PI3Ks are monomeric multi-domain proteins, which most clearly phosphorylate phosphatidylinositol (PtdIns) to produce PtdIns3P and also may generate PI(3,4)$P_2$ during endocytosis (Posor et al., 2013, Vanhaesebroeck et al., 2012). Class III PI3Ks are heterodimeric poteins, first identified in yeast. Their preferred substrate *in vivo* is PtdIns and they are mainly responsible for the production of most cellular PtdIns3P. Class I PI3Ks are the most heavily studied of all PI3Ks; they can catalyze the phosphorylation of PtdIns, PtdIns4P or PtdIns(4,5)$P_2$ *in vitro* but their preferred cellular substrate is PtdIns(4,5)$P_2$, leading to the synthesis of PtdIns(3,4,5)$P_3$, often known as $PIP_3$. Class I PI3K activity can stimulated rapidly (seconds) by extracellular signals through membrane receptors including RTKs (Receptor Tyrosine Kinases) and GPCRs (G-Protein coupled receptors) and has been heavily implicated in oncogenic transformation (Yuan and Cantley, 2008).

*Class I PI3Ks*

Class I PI3Ks are heterodimers, which consist of a catalytic subunit, p110 (four variants in humans, p110α, p110β, p110δ and p110γ) and one of a range of regulatory subunits (p85α, p85β, p55α, p50α, p55γ, p101 or p84) (Beck et al., 2014, Engelman, 2006, Stephens and Hawkins, 2011, Vanhaesebroeck, 1997a). Human Class I PI3Ks are further divided into two subclasses based on their subunit composition and mode of activation. In the Class IA enzymes, (p110α, p110β and p110δ, encoded by three different genes, PIK3CA, PIK3CB and PIK3CD respectively), the p110 subunit binds with an SH2 domain-containing regulatory subunit (p85α, p85β or p55γ, encoded by PIK3R1, PIK3R2 and PIK3R3 respectively, or p55α and p50α, splice variants of PIK3R1). These phospho-tyrosine binding SH2 domains allow regulation by Receptor Tyrosine Kinases, although p110β can be activated by either RTKs or G Protein Coupled Receptors (Maier et al., 1999, Tang and Downes, 1997). In contrast, class IB PI3K (p110γ) has a catalytic p110 subunit which binds to a p101 or p84 regulatory subunit and is activated by GPCRs. All of the p110 isoforms appear to be activated by direct interaction with small GTPases; in the case of p110$\alpha$, $\gamma$ and $\delta$, this appears to be RAS isoforms, whereas p110$\beta$ appears to be co-activated by RAC (Fritsch et al., 2013). Comparing genomic data implies evolutionary conservation of the PI3Ks, showing that one class I PI3K gene is present in *D. melanogaster*, *C. elegans* and three in *D. discoideum* (Engelman, 2006). However, the functional expansion provided by the four catalytic subunits appears to be restricted to vertebrates and some other chordates (Brown and Auger, 2011, Kawashima et al., 2003).

Class I PI3Ks are multi-domain proteins. Although all class I catalytic subunits are encoded by separate genes they share a similar domain organization, with, in order from the N-terminus, a p85 binding or Adapter binding domain (ABD specifically conserved in Class IA p110$\alpha$, $\beta$ and $\delta$), a Ras binding domain (RBD), a C2 domain, a helical domain (PIK) and a catalytic kinase domain at the C-terminus. The regulatory subunits of class IA all have a common core structure consists of p110 binding domain (also termed the iSH2) flanked by two Src-homology 2 (SH2) domains which bind to phospho-tyrosine residues in activated RTKs or adapter proteins and are thereby responsible for translocation to and activation of the PI3K holoenzyme at the plasma membrane (Burke and Williams, 2013, Vanhaesebroeck, 2012, Yu et al., 1998). The longer variants i.e. p85α and p85β, also contain an N-terminal SH3 domain and a BCR-homology (BH) domain with potential GTPase activating activity, flanked by two proline rich regions (Fruman et al., 1998).

*Isoform specific PI3K signalling*

The existence and differential expression of functionally diverged catalytic and regulatory subunits in different tissues or cell types suggests isoform specific signalling. p110α, p110β, p85α and p85β seem to be present ubiquitously with little evidence for selective heterodimeric pairing, but with some variation in levels of expression. p55γ has been reported to be restricted to liver cells, however expression of p110γ, p110δ, p101 and p84 is most evident in blood cells (Stephens and Hawkins, 2011, Vanhaesebroeck, 2001, Vanhaesebroeck et al., 1997b). The generation of isoform specific knock out (KO) mice, kinase dead mutant knock-in (KI) mice, work using microinjection of isoform specific anti-catalytic antibodies and isoform specific inhibitors has given much more insight into isoform specific signalling which has been reviewed well (Vanhaesebroeck et al., 2010a). Briefly, full deletion of either p110α or p110β leads to embryonic lethality and both enzymes appear to play important and distinctive roles in growth and development (Jia et al., 2008, Vanhaesebroeck, 2010a). Functional loss of p110$\gamma$ or $\delta$ on the other hand leads to impaired immune functions and has motivated the targeting of these enzymes for inflammatory disease (Hirsch et al., 2000, Okkenhaug et al., 2002, Sasaki et al., 2000).

**Phosphoinositides: ubiquitous eukaryotic signals**

PtdIns and its phosphorylated derivatives, including the lipid products of PI3Ks (class I, II and III) or other classes of PI kinases, are collectively called phosphoinositides (PIs) and are found throughout animals, plants, fungi and other eukaryotes that have been investigated (Michell, 2008). Structurally PIs are amphipathic molecules composed of D-*myo* inositol 1-phosphate polar head group, which faces the cytosolic side of the cell, attached via its phosphate group to diacylglycerol (DAG) which sits within the lipid bilayer, including two fatty acid chains which in mammals are most commonly found to correspond to arachidonic acid and stearic acid. The inositol head group can be phosphorylated at the 3$^{rd}$, 4$^{th}$ or 5$^{th}$ hydroxyl group, whereas the 2$^{nd}$ and 6$^{th}$ positions of inositol ring have not been known to become phosphorylated in phosphoinositide lipids. In metazoans, $PIP_3$, the lipid product of class I PI3Ks is known to act as an indispensable signal regulating diverse cellular processes with implications in both health and diseases. However, in yeast, although production of $PIP_3$ has been reported in *S. pombe* (Mitra et al., 2004) it appears to be detrimental to growth of *S. cerevisiae* (Rodriguez-Escudero et al., 2005). However other phosphoinositides including the class I PI3K substrate $PI(4,5)P_2$, appear to play important roles throughout the eukaryotes, including regulating cell

division and polarity in budding yeast (Bertin et al., 2010, Strahl and Thorner, 2007).

**Interpreting the language of lipids: PH domain containing PI3K effectors**

The class I PI3Ks influence cellular behavior through a diverse group of $PIP_3$-binding effector proteins (Fig. 1), in humans numbering at least 30 and possibly as many as 100. These effector proteins contain a lipid-binding domain in their structure with selective affinity for $PIP_3$, and in some cases causing bulk translocation of the protein onto the plasma membrane when $PIP_3$ levels rise. The most common lipid binding domain among various downstream effector proteins is the Pleckstrin Homology (PH) domain. The function most commonly attributed to PH domains has been this selective binding to phosphoinositide lipids (Haslam et al., 1993, Mayer et al., 1993), but importantly, analyses suggest that most do not possess this ability and play other roles (Lemmon, 2008, Yu et al., 2004). PH domains are evolutionary conserved (Lemmon, 2008) and approximately 120 amino acid long with, in the human genome, 323 examples of the domain reported in 276 different independent gene products (Leslie et al., 2012) but it is strongly expected that most have functions unrelated to phosphoinositides. The PH domains that have been shown to function in binding $PIP_3$ include the serine threonine kinases AKT and PDK1, protein tyrosine kinases of Bruton's tyrosine kinases (BTKs) and Tec family, the Cytohesins (GRP1/ARNO), and further more diverse GEFs (Guanine nucleotide Exchange Factors) and GAPs (GTPase Activating Proteins) for GTPases of the ras superfamily (Leslie, 2012, Park et al., 2008, Vanhaesebroeck, 2012).

*The AKT and PDK1 kinases*

The most heavily studied downstream mediators of PI3K function are the AKT kinases, also known as PKB (Protein Kinase B) which belong to the AGC family of Serine/Threonine kinases. They are activated in response to increased $PIP_3$ levels by two stable phosphorylation events. First, their PH domain-mediated translocation to the plasma membrane upon stimulated production of $PIP_3$ brings about a conformational change in the kinase. This in turn allows phosphorylation at the T308 position (numbering from human AKT1) in the activation loop by another PH domain-containing PI3K effector and protein kinase PDK1 (Alessi et al., 1997, Stephens et al., 1998) and at S473 position in the hydrophobic region of the C-terminal regulatory domain by TORC2 (Fayard et al., 2005, Sarbassov et al., 2005) which stabilizes the active conformation (Yang et al., 2002). Once activated, AKT can move into the cytoplasm or nucleus and phosphorylates a large number of substrates, leading to regulation of diverse cellular processes (Manning and Cantley, 2007, Toker and Marmiroli, 2014). In contrast to AKT, PDK1 is often

considered a constitutively active enzyme and it also activates several kinases other than AKT. Accordingly, the higher affinity of its PH domain for $PIP_3$ combined with its ability to bind to PtdSer ensure that a significant fraction of cellular PDK1 is located at the plasma membrane even in cells with very low levels of $PIP_3$ (Lucas and Cho, 2011, Mora et al., 2004).

There is strong evidence from genetics in mice, flies and worms and extensive characterization of signalling pathways in mammalian cells showing the key role of the AKT kinases in mediating many of the cellular effects of PI3K activation, particularly those on cell growth, proliferation and metabolism (Manning and Cantley, 2007, Toker and Marmiroli, 2014). However, many effects of PI3K on other processes, particularly migration and polarity, appear to be AKT independent (Charest and Firtel, 2007, Leslie et al., 2008, Liu et al., 2004). Perhaps the strongest evidence for the importance of AKT in mediating effects downstream of PI3K/PTEN is the demonstrated rescue of lethality caused by PTEN loss in drosophila that can be achieved with a mutation in dAKT which reduces the affinity of its PH domain for $PIP_3$ and which supports viability despite highly elevated $PIP_3$ levels (Stocker et al., 2002). However, flies appear to have far fewer $PIP_3$-binding PH domains than mammals (Park, 2008) and the relative significance of AKT downstream of PI3K/PTEN in humans is controversial, particularly given the commitment put into developing drugs targeting the AKT/TOR signalling axis to treat cancers with activated PI3K or lacking PTEN.

*The Cytohesin family of ARF-GAP proteins*

The established PH domain containing $PIP_3$-binding proteins also include the cytohesin family of proteins, consisting of four members: cytohesin-1, cytohesin-2 (ARNO, Arf nucleotide binding site opener), cytohesin-3 (GRP1, general receptor for phosphoinositides) and cytohesin-4. All the members of the cytohesin family contain a N-terminal coiled coil (CC) domain, a central Sec7 domain and a C-terminal PH domain. The Sec7 domain possesses a highly conserved Arf-GEF function which mediates the activation of Arf GTPases (Chardin et al., 1996). All the cytohesin protein PH domains appear to mediate recruitment to the plasma membrane and thus can allow regulation by PI3K (Klarlund et al., 1997, Nagel et al., 1998, Venkateswarlu et al., 1998).

*Other PI3K effectors*

Many further class I PI3K effector proteins, such as FGD6, were identified by functional and/or bioinformatic screening of PH-domains (Dowler et al., 2000, Park, 2008). However some unbiased proteomic approaches to identify other phosphoinositide lipid binding domains have been highly successful, leading to the discovery of further PH domain containing proteins, such as the ARAP

proteins (Jungmichel et al., 2014, Krugmann et al., 2002), and others, with apparently novel $PIP_3$ and $PI(3,4)P_2$ binding domains (Dixon et al., 2011, Dixon et al., 2012, Lee et al., 2005, Lemmon, 2008, Premkumar et al., 2010).

**Phosphoinositide phosphatases**

The major cellular lipid product of class I PI3Ks, $PIP_3$ is metabolized by various 3-, and 5- phosphatases at these positions of the inositol ring respectively. A major antagonist of PI3K signaling is the 3-phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) which converts $PIP_3$ back to its more abundant precursor $PI(4,5)P_2$ (Cantley and Neel, 1999, Downes et al., 2001, Stambolic et al., 1998). The 5-phosphatases SHIP1/2 (and probably also SHIP) also metabolize $PIP_3$, converting it to the alternate signal $PI(3,4)P_2$ which itself contributes to mediating the outcomes of PI3K activation (Leslie, 2012). The contribution of $PI(3,4)P_2$ in mediating the outcomes of PI3K activation is currently unclear but some evidence, such as the tumour suppressor status of INPP4B, a 4-phosphatase which dephosphorylates $PI(3,4)P_2$ (Gewinner et al., 2009), implies important roles.

*PTEN: a phosphoinositide 3-phosphatase and tumour suppressor*

In 1997, two independent research groups first identified PTEN as a candidate tumor suppressor (Li et al., 1997, Steck et al., 1997). It exists predominantly as a 403 amino acid protein as well as a very interesting but only recently identified 576 amino acid secreted isoform, is a member of PTP (Protein Tyrosine Phosphatase) family and in vitro has phosphatase activity against both protein and lipid substrates (Hopkins et al., 2013, Worby and Dixon, 2014). However, its principle substrate appears to be $PIP_3$ (McConnachie et al., 2003, Salmena et al., 2008). A key determinant of the tumour suppressor activity of PTEN seems to be that under normal cellular conditions, the PTEN protein pool has substantial constitutive activity keeping $PIP_3$ levels tightly regulated and maintaining homeostasis (Leslie and Foti, 2011, Myers et al., 1998, Stambolic, 1998). The *PTEN* gene has long been known to be the subject of mutation and deletion in cancer, causing loss of tumour suppressor function (Bonneau and Longy, 2000, Salmena, 2008). However, PTEN levels and it's activity are controlled at the transcriptional, translational and post-translational levels (Bertrand et al., 2014, Leslie, 2008) and significantly, evidence has more recently accumulated showing that the physiological mechanisms that control PTEN activity are themselves dysregulated in cancer, leading indirectly to loss of PTEN function (Leslie and Foti, 2011, Poliseno et al., 2010, Silva et al., 2008).

*5-phosphatase SHIP proteins*

The phosphoinositide 5-phosphatases are a large family in mammals of 10 $Mg^{2+}$-dependent phosphatases including OCRL, INPP5E, SKIP, SHIP1 and SHIP2. Many of them are catalytically active and capable of metabolizing $PIP_3$ (Astle et al., 2007, Ooms et al., 2009), however SHIP1 (hematopoietic restricted) and SHIP2 (more widely expressed) have most clearly been demonstrated to regulate PI3K-dependent signalling in cells. Unlike PTEN which appears to provide constant activity, the activity of both SHIP1 and SHIP2 is increased by tyrosine phosphorylation and regulated membrane recruitment (Batty et al., 2007, Rohrschneider et al., 2000). The large 145kDa SHIP proteins contain a N-terminal SH2 domain, which helps bind SHIP to tyrosine phosphorylated forms of Shc, SHP-2, Doks, certain ITIMs (immunoreceptor tyrosine based inhibitory motifs) and ITAMs (immunoreceptor tyrosine based activation motifs). In addition, SHIP proteins contain a centrally located catalytic phosphatase domain, followed by a C2 domain, potentially involved in allosteric regulation by PtdIns(3,4)$P_2$ and two NPXY motifs and four PxxP motifs at C-terminus which are involved in protein-protein interactions (Dyson et al., 2012, Rohrschneider, 2000).

**The evolution of the Class I PI 3-Kinase signalling network**

Class I PI 3-Kinases were discovered and characterized in vertebrates acting at the inner leaflet of the plasma membrane, allowing the cells of these multicellular organisms to interpret intercellular signals such as hormones, growth factors and neurotransmitters (Downes and Carter, 1991, Parker and Waterfield, 1992). Since then, the roles of these PI3Ks in the regulation of cell proliferation, growth and survival in response to intercellular communication has been intensely studied and conserved functions in invertebrates such as flies and worms have been established (Engelman, 2006). However, genome sequence analysis can help identify key components of the signalling network, including class I PI3K enzymes themselves, in many single celled organisms. The results imply that the network must have existed in a single celled common ancestor in which it fulfilled somewhat different functions. Notably, in animals, the identification of localised pools of $PIP_3$ in the leading edge of cells such as neutrophils undergoing chemotaxis, in axonal growth cones during neuronal polarization and in the basolateral membranes of some

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and Downward, 1999, Weiner, 2002). Similar polarization of PI3K signalling has been observed during directed cell migration in the protozoan Dictyostelium discoideum (Charest and Firtel, 2006, Iijima et al., 2002, Weiner, 2002). Therefore, it is tempting to speculate that the ancestral role for

class I PI3K may perhaps relate to chemotaxis and feeding and the linked remodeling of metabolism in response to nutrient availability. Here we present and discuss an analysis of recent available genome sequence information.

## Eukaryotic conservation of PI kinase and phosphatases

The genomes of most groups of single celled organisms and perhaps all multicellular organisms contain both phosphoinositide kinases with similarity to the class I PI3Ks and cysteine dependent phosphoinositide phosphatases related to PTEN. These extensively conserved enzymes are most similar to the related human phosphoinositide 4-kinases and class III PI 3-kinases (the latter also known as PIK3C3 or VPS34) and to the Myotubularin and Sac groups of PI phosphatases. In terms of apparent evolutionary distance from humans, each of the enzyme groups is observed in most eukaryotic genomes, including fungi, amoebozoa, plants, the chromalveolata group (including alveolates and stramenopiles and rhizaria) and excavates such as giardia.

### *Enzymes of $PIP_3$ synthesis and metabolism*

Class I PI3K catalytic subunits sequentially contain a Ras-binding domain, a C2 domain and a C-terminal PI-kinase domain and proteins with this domain structure and sequence similarity closest to class I enzymes are found throughout many unikonts, including amoebozoa, fonticula, choannoflagellates and animals, with the notable exception of the fungi. Functional studies provide confidence that these unikont enzymes represent true class I PI3Ks (Clark et al., 2014) supported by phylogenetic analyses (Brown and Auger, 2011) and are shown in Fig. 2. Class I PI3Ks synthesize $PIP_3$ from $PI(4,5)P_2$ and are related to the class II PI3K enzymes which synthesize PI3P and in some cases $PI(3,4)P_2$ (Posor, 2013, Vanhaesebroeck, 2012). However, the Class I and II PI3K catalytic subunits display extensive structural similarity, with the class II enzymes also sequentially containing a Ras-binding domain, a C2 domain and a C-terminal PI-kinase domain, making the two groups difficult to distinguish from amino acid sequence without functional data. In more distantly related phylogenetic groups, although such class I/II PI3Ks appear to be absent from prokaryotes, from many excavates and stramenopiles and from almost all plants, they are present in many other diverse eukaryotic groups. These PI3Ks are found in several parasitic excavates such as kinetoplastid trypanosomes, trichomonas, naegleria and giardia (Cox et al., 2006) and examples are also identifiable in the genomes of several alveolates (eg Tetrahymena thermophila, XP_001022838; Ichthyophthirius multifiliis, XP_004025156; Paramecium tetraurelia, XP_001451414) and a few stramenopiles (eg. Albugo candida, CCI1092; Phytophthora infestans, XP_002905219). Without functional data, their firm functional classification into class I or class II is difficult, but comparisons with

class I enzyme sequences including Dictyostelium discoideum PI3Ks finding modestly greater similarity to these divergent eukaryote PI3Ks and the acutely regulated synthesis of $PIP_3$ in Dictyostelium (Clark, 2014) imply that class I PI3K signalling may be more broadly distributed than previously proposed. It is difficult to distinguish whether this broad distribution of Class I/II enzymes in more distant eukaryotes indicates loss from specific groups (as seems likely for the fungi) or (perhaps less likely given the diversity of groups identified) gain by horizontal transfer into specific groups such as the excavate parasites (See Fig. 2).

The Class IA regulatory subunits with some similarity to p85, defined here by tandem phosphotyrosine-binding SH2 domains and p110-binding inter-SH2 region, are only evident in animals and close relatives, with the most evolutionarily distant protein with this retained domain architecture currently identified in the choanoflagellate Salpingoeca rosetta. This is in accordance with the distribution of tyrosine kinase based regulatory systems which are absent from many other eukaryotic groups. The expansion of the catalytic p110 gene family into class 1A and 1B enzymes is evident only in the vertebrates and other chordates (eg Ciona intestinalis (Kawashima, 2003)), coinciding with the appearance of the class 1B adaptors p101 and p87 which lack clear relatives in nematodes or insects.

Phosphatases similar to the human $PIP_3$ 3-phosphatase, PTEN, sharing its tightly associated Phosphatase-C2 domain architecture are found throughout eukaryotes, including fungi, plants, stramenopiles, alveolates and rhizaria and euglenozoa. Since some of these organisms lack class I PI3Ks, it would appear that some PTEN-like enzymes may function to utilize an alternate substrate, or possibly to ensure $PIP_3$ produced by other routes does not accumulate, as in some organisms, such as Saccharomyces cerevisiae, PI3K activity is strongly deleterious (Rodriguez-Escudero, 2005). On this point it is also relevant that in chordate genomes, the closest protein relative of PTEN, VSP/TPIP is able to metabolise several phosphoinositides, acting both as a 3- and 5-phosphatase (Kurokawa et al., 2012, Walker et al., 2001). Additionally, the SHIP group of $PIP_3$ 5-phosphatases, including an N-terminal SH2 domain, show conservation within animals, choanoflagellates and the related Capsaspora, but are not evident in other groups (most of which lack phosphotyrosine based signalling mechanisms). Given the existence of many phosphoinositide 5-phosphatases with substrates other than $PIP_3$, it is difficult from sequence analysis to predict whether other phosphotyrosine-independent $PIP_3$ 5-phosphatases may function in more distantly related organisms.

*Functional conservation of $PIP_3$ signalling – the evolution of $PIP_3$-binding proteins*

Attempts to analyse the evolutionary diversity of PIP3-binding proteins are not simple, as the family of domains most commonly known to function as $PIP_3$-binders, the pleckstrin homology (PH) domains, also contains many examples that lack this capability and in some cases bind to other lipids. In the absence of solid functional data, prediction of binding characteristics has proved difficult. Therefore, in analyses based on simple sequence similarity and retained domain architecture, both the AKT and Cytohesin groups of $PIP_3$-binding effector proteins show a similar evolutionary distribution to the class I PI3K enzymes in unikonts but not fungi, implying highly conserved functions for these proteins (Fig. 3). Although these conserved functions are well recognized for the AKT kinases (Manning and Cantley, 2007, Paradis and Ruvkun, 1998, Stocker, 2002), the functions of the cytohesins are less clearly defined.

Proteins structurally related to the human $PIP_3$ binding kinase PDK1 are found in many more distant groups of eukaryotes, including plants and fungi which lack class I PI3Ks. However the recognized binding of the PDK1 PH domain to phosphatidylserine (Lucas and Cho, 2011) and the enzyme's role phosphorylating and activating many $PIP_3$-independent protein kinases implies PI3K-independent conserved functions. Similarly, the DH-PH domain pair, shared by several $PIP_3$-binding Guanine nucleotide exchange factors for the Rac/cdc42 group of GTPases, is also found in many other proteins without apparent $PIP_3$-binding capability and is found in many branches of eukaryotes.

The apparent evolutionary conservation of PI3K pathway components seems likely to reflect conserved functions for these proteins. Studies in invertebrate model organisms, particularly flies and worms, have already contributed greatly to our understanding of the regulation of cell growth, proliferation and movement but further fundamental questions remain unanswered regarding the regulation of these processes and the roles of the PI3K network. Further motivation for such studies is provided by the availability of drugs to inhibit specific PI3K isoforms and some downstream PI3K-activated proteins such as AKT and TOR (Martelli et al., 2014, Vanhaesebroeck et al., 2010b). A deeper understanding of the underlying biology should help greatly in the successful trialing and application of these new therapies.

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**Figure Legends**

**Fig. 1.** Class I PI3K function. The diagram shows a model of PI3K function, activated by a receptor at the plasma membrane. Upon receptor engagement PtdIns(4,5)$P_2$ (PI(4,5)$P_2$) is phosphorylated by activated PI3K and ATP to form PtdIns(3,4,5)$P_3$ ($PIP_3$). and the reverse conversion catalyzed by PTEN, reproducing PI(4,5)$P_2$ and releasing free phosphate. Once generated PIP3 is able to influence diverse cellular processes through a large number of direct $PIP_3$-binding proteins, including AKT, activating Guanine nucleotide exchange factors (GEFs) for small GTPases, PDK1, the Cytohesins and many others.

**Fig. 2**. Eukaryotic phylogenetic tree and the PI3K system. A simplified representation of eukaryotic evolutionary relationships is presented. Several aspects, particularly the relationships of the deepest branches are uncertain (Nozaki et al., 2009, Parfrey et al., 2010, Rogozin et al., 2009). The evolutionary distances represented in the tree by branch lengths are indicative but do not represent a single quantified analysis. Proteins structurally and functionally resembling the human class I PI3Ks are present throughout the Unikonts (Episthokonts and Amoebozoa) with the notable exception of fungi. PI3K enzymes are also present in the genomes of several more divergent eukaryotes (eg some excavate parasites, including trypanosomes and giardia, and some alveolates and stramenopiles) but without functional characterisation they are difficult to classify into the class I or class II PI3K groups. Both the regulatory p85 PI3K subunits and SHIP 5-phosphatases appear to be a more recent evolutionary development, appearing only in metazoa and chaonnoflagellates.

**Fig. 3**. Occurrence of PI3K pathway components in eukaryotic groups. The patterns of occurrence of individual components of the PI3K signalling system is shown, relating to the phylogenetic tree shown in Fig. 2. Lineages or species are shown in pink shading, in which each protein represented below is evident. It should be noted (*) that proteins with similarity to class I p110 PI3K are present in some species from the more diverse lineages noted, but it is not clear whether these represent class I or class II enzymes. Otherwise a pattern of distribution is shred between p110, AKT and the cytohesins, being found in unikont groups other than fungi. Also, the Giardia intestinalis genome contains a phosphatase with sequence similarity to the PTEN catalytic domain but lacking evident amino acid sequence or 3D structural alignment to the adjacent C2 domain, which has been used here as the defining feature of the PTEN enzyme group.

Fig. 1

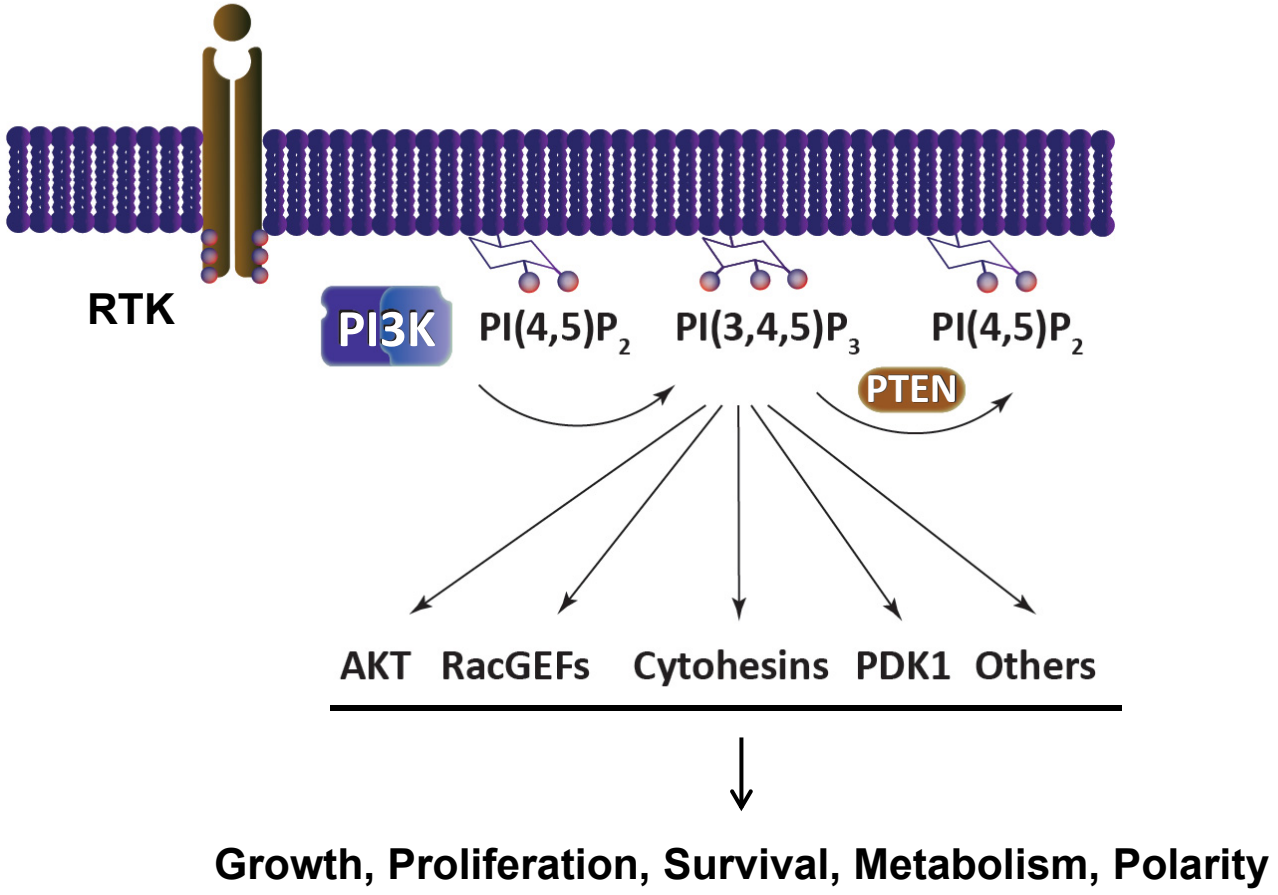

RTK
PI3K
PI(4,5)P2
PI(3,4,5)P3
PI(4,5)P2
PTEN
AKT
RacGEFs
Cytohesins
PDK1
Others
Growth, Proliferation, Survival, Metabolism, Polarity

Fig. 2

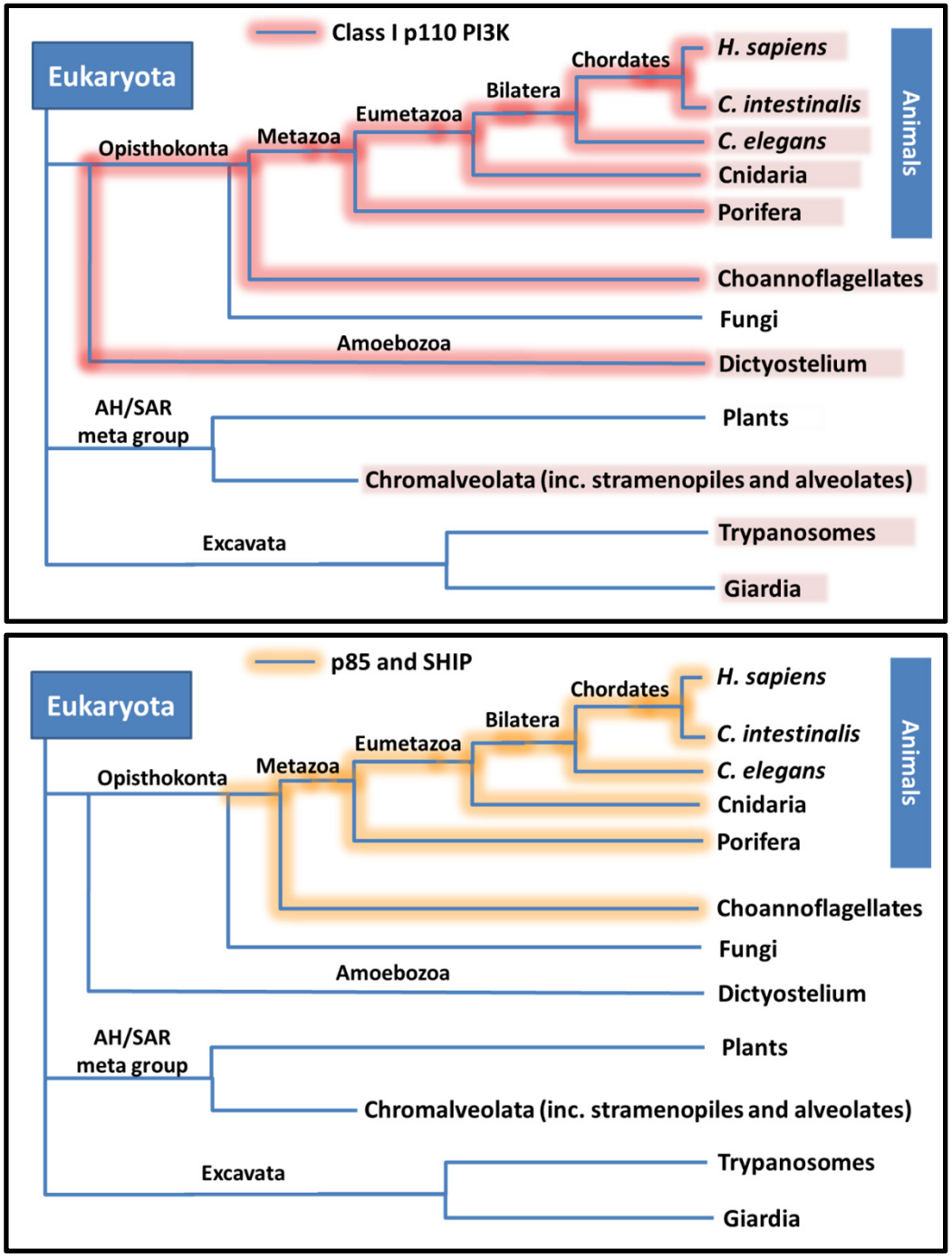
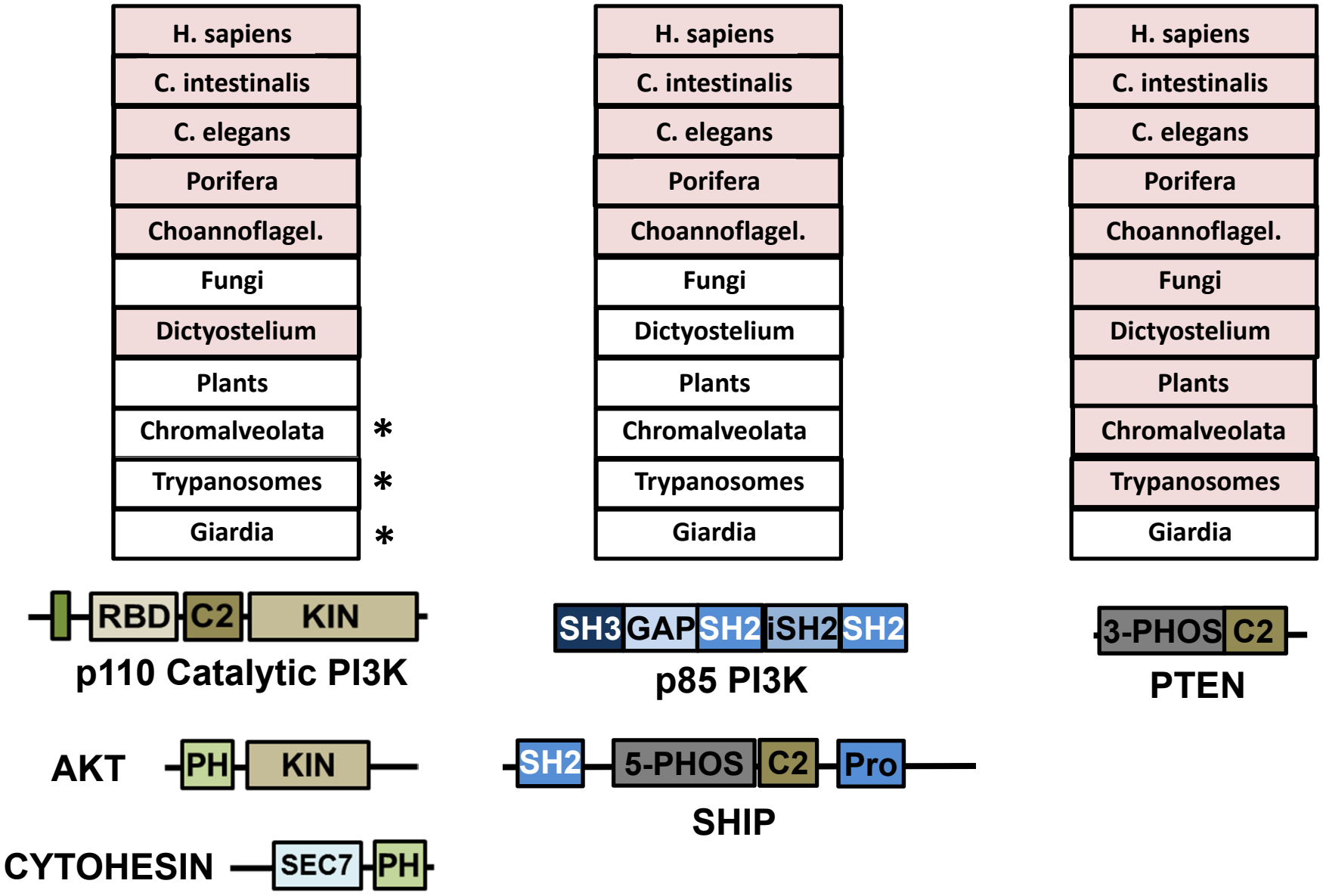

Class I p110 PI3K
Eukaryota
Chordates
Bilatera
Eumetazoa
Metazoa
Opisthokonta
H. sapiens
C. intestinalis
C. elegans
Cnidaria
Porifera
Animals
Choannoflagellates
Fungi
Amoebozoa
Dictyostelium
AH/SAR
meta group
Plants
Chromalveolata (inc. stramenopiles and alveolates)
Excavata
Trypanosomes
Giardia
p85 and SHIP
Eukaryota
Chordates
Bilatera
Eumetazoa
Metazoa
Opisthokonta
H. sapiens
C. intestinalis
C. elegans
Cnidaria
Porifera
Animals
Choannoflagellates
Fungi
Amoebozoa
Dictyostelium
AH/SAR
meta group
Plants
Chromalveolata (inc. stramenopiles and alveolates)
Excavata
Trypanosomes
Giardia

Fig. 3


H. sapiens
C. intestinalis
C. elegans
Porifera
Choannoflagel.
Fungi
Dictyostelium
Plants
Chromalveolata
Trypanosomes
Giardia
*
*
*
RBD
C2
KIN
p110 Catalytic PI3K
SH3
GAP
SH2
iSH2
SH2
p85 PI3K
3-PHOS
C2
PTEN
AKT
PH
KIN
SH2
5-PHOS
C2
Pro
SHIP
CYTOHESIN
SEC7
PH